PATENT COOPERATION TREATY

by fax and post

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

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B'arb.: F: 15.02.01
2. Feb. 2001
25.03
VFrist: Frist 29.4.01
Vfg:

PCT

WRITTEN OPINION

(PCT Rule 66)

Date of mailing *(day/month/year)*	29.01.2001
Applicant's or agent's file reference: 3377/99 PCT	**REPLY DUE** **within 3 month(s)** from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/02701	28/03/2000	01/04/1999

International Patent Classification (IPC) or both national classification and IPC
C12N15/54

Applicant
BASF PLANT SCIENCE GmbH et al.

1. This written opinion is the **first** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☒	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☒	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☒	Certain document cited
VII	☒	Certain defects in the international application
VIII	☒	Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 01/08/2001.

Name and mailing address of the international preliminary examining authority:
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Authorized officer / Examiner
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I. Basis of the opinion

1. This opinion has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".)*:

Description, pages:

32	as originally filed			
1-31	as received on	15/09/2000	with letter of	12/09/2000

Claims, No.:

1-27	as received on	15/09/2000	with letter of	12/09/2000

Drawings, sheets:

1/6-6/6	as received on	15/09/2000	with letter of	12/09/2000

Sequence listing part of the description, pages:

1-45 (SEQ ID NOs. 1-15), as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☒ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☒ furnished subsequently to this Authority in computer readable form.
   - ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☒ The statement that the information recorded in computer readable form is identical to the written sequence

listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:
   ☐ the claims, Nos.:
   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 22 (partially),

because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☒ the claims, or said claims Nos. 22 (partially) are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A written opinion cannot be drawn due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.
   ☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation (Form PCT/IPEA/405) to restrict or pay additional fees, the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with for the following reasons and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees:
   see separate sheet

3. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this opinion:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrialapplicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-27: NO
Inventive step (IS)	Claims	1-27: NO
Industrial applicability (IA)	Claims	

2. Citations and explanations
   see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

   see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

The application concerns the provision of a yeast and plant polypeptide and polynucleotide sequences allegedly corresponding to diacylglycerol acyltransferases. Function is shown for *Saccharomyces cerevisiae* sequences, but neither the function nor any structural relationship to the *Saccharomyces* sequences making such a function plausible are demonstrated for the other full-length and partial sequences.

Re Item II
Priority

After considering the priority document, the documents cited "P, X" in the search report are not considered relevant for the examination of novelty and inventive step.

Re Item III
Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claim 22 seeks protection for cells or organisms with altered PDAT activity, "wherein the altered PDAT activity is characterized by an alteration in gene expression, catalytic activity and/or regulation of activity of the enzyme". No reference could be found in the description for alterations to the catalytic activity or regulation of PDAT activity and claim 22 (partially) is therefore to lack meaningful support from the description. The claim has only been examined with respect to alterations in gene expression.

Re Item IV
Lack of Unity of Invention

An international application must relate to one invention only or to a group of inventions so linked as to form a single general inventive concept. Unity of invention is fulfilled only when there is a technical relationship between the inventions involving one or more of the same or corresponding special technical features. Special technical features are such features that define the contribution of the claimed

invention over the prior art.

The identified 8 inventions relate to a group of sequences with the claimed technical feature of being diacylglycerol acyltransferases as the sole common link. However, this feature cannot be considered to constitute a special technical feature because it does not define a contribution over the prior art: SEQ ID NOs. 2, 3, 9, 16, 20 and 22 have all been previously disclosed in their entirety (D1, D2 and D3). The application therefore does not meet the requirements of Rule 13.2 PCT in that there is no common special technical feature linking the 8 inventions of the application, these being:

Invention I Claims 1, 3, 6, 7, 9, 11-27 (all partially) 2 and 4 (completely)
Enzymes catalysing the acyl-CoA-independent transfer of fatty acids to diacylglycerol in the production of triacyglycerol from *Saccharomyces cerevisiae* and corresponding to polypeptides with SEQ ID NOs. 2, 16, 20 and 22, encoded by polynucleotides SEQ ID NOs. 1, 19 and 21, fragments, derivatives, alleles, homologs and isoenzymes, the corresponding polynucleotide sequences, portions, derivates, alleles and homologs of the polynucleotide sequence, expression vectors, transgenic cells and organisms, processes for the production of triacylglycerol using such cells/organisms, the product of such a process and the use of the enzymes and polynucleotides in such processes.

Invention II Claims 1, 3, 5-9 and 11-27 (all partially)
As invention I with SEQ ID NOs. 3, 13 and 23 from *Schizosaccharomyces pombe*.

Invention III Claims 1, 3 and 5-27 (all partially)
As invention I with SEQ ID NOs. 4-6, 18, 24, 25 from *Arabidopsis thaliana*.

Invention IV Claims 1, 3 and 5-27 (all partially)
As invention I with SEQ ID NOs. 7, 8, 26 and 27 from *Zea mays*.

Invention V Claims 1, 3, 6-8 and 10-27 (all partially)
As invention I with SEQ ID NOs. 9 and 28 from *Neurospora crassa*.

Invention VI Claims 1, 3, 5-9 and 11-27 (all partially)
As invention I with SEQ ID NOs. 10, 14, 17 and 29 from *Arabidopsis thaliana.*

Invention VII Claims 1, 3, 5-9 and 11-27 (all partially)
As invention I with SEQ ID NOs. 11, 15 and 30 from *Arabidopsis thaliana.*

Invention VIII Claims 1, 3 and 5-27 (all partially)
As invention I with SEQ ID NOs. 12 and 31 from *Lycopersicon esculentum.*

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1) **Reference is made to the following documents:**

D1: PETER VERHASSELT ET AL.: 'Twelve open reading frames revealed in the 23.6kb segent flanking the centromere on the Saccharomyces cerevisiae chromosome XIV right arm' YEAST, vol. 10, no. 7, July 1994 (1994-07), pages 1355-1361, XP002112572 -& Swissprot Database Entry Yn84_Yeast Accession number P40345; 1 February 1995 XP002112574

D2: DATABASE EMBL [Online] Database Entry SPBC776, 21 January 1999 (1999-01-21) LYNE M. ET AL.: 'S. pombe chromosome II cosmid c776' Database accession no. AL035263 XP002150203

D3: DATABASE EMBL [Online] Database Entry AI398644, 10 February 1999 (1999-02-10) XP002150204 & MARY ANNE NELSON ET AL.: 'Expressed sequences from conidial, mycelial, and sexual stages of Neurospora crassa ' FUNGAL GENETICS AND BIOLOGY, vol. 21, 1997, pages 348-363, XP000952173

D4: KEITH STOBART ET AL.: 'Triacylglycerols are synthesized and utilized by transacylation reactions in microsomal preparations of developing safflower (Carthamus tinctorius L.) seeds' PLANTA, vol. 203, no. 1, 1997, pages 58-66, XP002112573

D5: WO 98 55631 A (CALGENE LLC) 10 December 1998 (1998-12-10)

2) **Novelty - Art.33(1) and (2) PCT:**

Invention I Claims 1, 3, 6, 7, 9, 11-27 (all partially) 2 and 4 (completely)

Claims 1, 3, 6 (partially), 2 and 4 (completely) lack novelty in light of the sequence provided by D2 which, according to the description, encodes an acyl-CoA-independent acyltransferase.

Claims 7-9 and 11 (partially) also lack novelty in light of D1 and D3, which disclose the *S. pombe* and *N. crassa* polynucleotide sequences respectively. These sequences are considered to be homologs of (claim 9) or homologous to (claim 11) SEQ ID NO. 1.

Claims 12-24, 26 and 27 (partially) cannot presently be acknowledged as being novel. The said claims are dependent on claims 1-4 and 6-11, which are formulated in such a vague and broad manner that dependent subject matter cannot be considered novel. Until the subject matter of the said claims is clarified, it will not be possible to acknowledge novelty; it is not possible, for example, to acknowledge as novel "a gene construct comprising a nucleotide sequence according to claims 7 to 11 operably linked to a heterologous nucleic acid", where the nucleic acid of claim 9 includes portions, derivates, alleles or homologs of the claimed sequences and are thus not adequately defined. See also Item VIII a) below.

If the Applicant were able to restore novelty to claims 1-4 and 6-11 (partially), it appears that the dependent claims 12-24, 26 and 27 (partially) could be acknowledged as being novel.

Claim 25 (partially) lacks novelty in light of D4, which discloses triacylglycerol made with an acyl-Co-A independent acyltransferase (D4 page 59 left-hand column paragraph 1). Even if the claim were restricted to triacylglycerol made using novel subject matter, the Applicant would need to show how this product differs from previously disclosed subject matter, as a product is not rendered novel by a new method for making it.

Inventions II-VIII Claims 1, 3 and 5-27 (all partially)

Claims 1, 3 and 7-11 (partially) lack novelty in light of the sequences provided by D1, D2 and D3 which, according to the description, are polypeptides and polynucleotides corresponding to phospholipid:diacylglycerol acyltransferases. Identifying the function of known polypeptides does not render the polypeptides novel.

Claims 5 and 6 (partially) cannot be acknowledged as being novel. The sequences disclosed in D1, D2 and D3 are considered to be homologs and/or isoenzymes of the claimed subject matter. Furthermore, without any further definition, fragments of the said enzymes can be construed as referring to individual amino acids or even smaller components.

Claims 12-24, 26 and 27 (partially) cannot be acknowledged as being novel. The said claims are dependent on claims 1-4 and 6-11, which are formulated in such a vague and broad manner that dependent subject matter cannot be considered novel. Until the subject matter of the said claims is clarified, it will not be possible to acknowledge novelty; it is not possible, for example, to acknowledge as novel "a gene construct comprising a nucleotide sequence according to claims 7 to 11 operably linked to a heterologous nucleic acid", where the nucleic acid of claim 9 includes portions, derivates, alleles or homologs of the claimed sequences and are thus not adequately defined. See also Item VIII a) below.

Claim 25 (partially) lacks novelty in light of D4, which discloses triacylglycerol made with an acyl-Co-A independent acyltransferase (D4 page 59 left-hand column paragraph 1). Even if the claim were restricted to triacylglycerol made using novel subject matter, the Applicant would need to show how this product differs from previously disclosed subject matter, as a product is not rendered novel by a new method for making it.

3) **Inventive Step - Art.33(1) and (3) PCT:**

The following comments on inventive step are confined to subject matter which could

be acknowledged as being novel, or for which novelty could potentially be restored as outlined *supra*.

Invention I Claims 1, 3, 6, 7, 9, 11-27 (all partially) 2 and 4 (completely)

The closest prior art is document D5, which discloses a the polypeptide and polynucleotide sequences for an acyl-Co-A dependent plant diacylglycerol acyltransferase as well as the use of the sequences in engineering plants with altered triacylglycerol content (D5 page 3 line 22 to page 5 line 20).

In the light of the prior art, the technical problem can be regarded as the provision of further polynucleotide and polypeptide sequences encoding enzymes that can alter the triacylglycerol content of cells or organisms expressing them.

If novelty were to be restored to the claims of Invention I, it appears that it would be possible to acknowledge inventive step for parts of the application, as the specific use of the polypeptide and polynucleotide sequences of the application has neither been disclosed not suggested.

Inventions II-VIII Claims 1, 3 and 5-27 (all partially)

Again, the closest prior art is document D5, which discloses a the polypeptide and polynucleotide sequences for an acyl-Co-A dependent plant diacylglycerol acyltransferase as well as the use of the sequences in engineering plants with altered triacylglycerol content (D5 page 3 line 22 to page 5 line 20).

In the light of the prior art, the technical problem can be regarded as the provision of further polynucleotide and polypeptide sequences encoding enzymes that can alter the triacylglycerol content of cells or organisms expressing them.

Even if novelty were to be restored to the claims of Inventions II-VIII through a narrowing of the claims, it cannot be seen how inventive step can be recognised. Although function has been demonstrated for the enzyme encoded by SEQ ID NO.

1, no such function has been demonstrated for the sequences from other species, nor has the Applicant shown that there is a structural relationship between the sequences of Invention I and those of Inventions II-VIII that would make such a function plausible. This is true for the full-length sequences as well as the partial sequences disclosed in the application.

4) **Requirements for any Amendments Art. 34(2)(b) PCT:**

Any information the applicant may wish to submit concerning the subject-matter of the invention, for example further details of its advantages or of the problem it solves, and for which there is no basis in the application as filed, should be confined to the letter of reply and not be incorporated into the application.

In order to facilitate the examination of the conformity of the amended application with the requirements of Article 34(2)(b) PCT, the applicant is requested to clearly identify the amendments carried out, no matter whether they concern amendments by addition, replacement or deletion, and to indicate the passages of the application as filed on which these amendments are based (see also Rule 66.8(a) PCT).

Re Item VII
Certain defects in the international application

a) Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1-D5 are not mentioned in the description, nor are these documents identified therein.

Re Item VIII
Certain observations on the international application

a) Several claims fail to meet the requirements of Article 6 PCT in that they do not disclose the subject matter in a sufficiently clear manner. These include:
(i) Claims 2-6 seek protection for "functional fragments, derivates, alleles,

homologs or isoenzymes" of the disclosed subject matter. However, it is not possible to determine from the claims exactly what the scope of this subject matter is, as the terms are not defined in the claims (e.g. the length or the precise function of the "functional fragment", extent of sequence identity of the "homolog", technical features defining "isoenzyme").

(ii) Claims 6, 9 and 10 similarly seek protection for portions, derivates, alleles or homologs of polynucleotide sequences without enabling the skilled artisan to determine the extent of the claim.

(iii) Claim 11 seeks protection for sequences which are "40% homologous" to sequences of the application. The term "homologous" should be replaced with "identical", as homology is not an absolute function.

(iv) Claims 19-22 seek protection for plants or cells with altered biosynthetic pathways, oil content or PDAT activity, without defining the alteration. The terms should be more clearly defined.

(v) Similarly, claim 23 seeks protection for plants or cells in which the accumulation of "undesirable fatty acids" is prevented, without defining what these fatty acids are, or technical features characterising the desired alteration.

(vi) Claim 26 seeks protection for a method leading to the production of triacylglycerols with uncommon fatty acids, without defining "uncommon fatty acid".

b) Claims 1, 3, 7 and 8 lack clarity (Article 6 PCT): The Applicant is reminded that the claims must be comprehensible from the technical point of view and clearly define the object of the invention, that is to say indicate all the essential features thereof (Rule 6 PCT). The subject-matter of the said claims does not fulfil this condition, as the claimed enzymes and nucleic acids are only defined by the function or name of the enzyme without disclosing any technical feature which unambiguously characterizes the claimed subject-matter. Enzymes and genes, being a chemical products should be clearly defined by their formula i.e. its amino acid or nucleotide sequence.

c) Several of the SEQ ID NOs. appear to be identical duplicates of each other, resulting in a lack of conciseness as required by Article 6 PCT. The unnecessary duplicates should be removed.